International Journal of Medicine
and Pharmaceutical Science (IJMPS)
ISSN (P): 2250-0049; ISSN (E): 2321-0095
Vol. 12, Issue 1, Jun 2022, 87–94

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF *ANNONA MURICATA L.* EXTRACTS

SHUBHA K. S[1] & USHA S[2]

[1]*Department of Microbiology, Cauvery Institute of Allied Health Sciences, Mysuru, Karnataka, India*

[2]*Department of Biochemistry, Cauvery Institute of Allied Health Sciences, Mysuru, Karnataka, India*

ABSTRACT

Medicinal plants continue to play an important part in satisfying people's basic health care needs all around the world. Plant extracts are used to treat a variety of ailments. Annona muricata L, an essential medicinal plant used to treat cancer, malaria, neuralgia, arthritis, diarrhoea, dysentery, and other conditions, was chosen for testing its antioxidant capacity utilising in vitro methods such as DDPH, ABTS, and the Nitric oxide radical scavenging assay. The methanol extract of Annona muricata L had the best antioxidant activity, with suppression of DPPH and ABTS radicals of 85.70 percent (IC50=0.127 mg/mL) and 98.60 percent (0.130 mg/mL) respectively. The chloroform extract of Annona muricata L showed maximum of47.36% nitric oxide (NO) inhibition with IC50 being 0.856 mg/mL with the tested concentrations (100-500 µg). When compared to other extracts, the methanol extract of Annona muricata L was very efficient against free radical–mediated illnesses.

KEYWORDS: Annona Muricata L extracts, Antioxidant Activities, DPPH, ABTS & Nitric Oxide Radicals

Received: Mar 19, 2022; **Accepted**: Apr 09, 2022; **Published**: May 07, 2022; **Paper Id.**: IJMPSJUN202209

Original Article

1. INTRODUCTION

ROS produces oxidative damage to bio molecules, resulting in a variety of pathological processes including atherosclerosis, cancer, cardiovascular dysfunction, autoimmune illness, neurological diseases, liver disease, and ageing [13]. ROS are highly unstable molecules that easily combine with other molecules to generate a stable compound, rendering them unstable [3]. These compounds are created both endogenously and exogenously by pollutants, chemicals, and ionising radiation. Antioxidants can neutralise free radicals like superoxide and hydroxyl radicals, as well as non–free radical species including hydrogen peroxide and singlet oxygen [7]. Antioxidants are chemicals that scavenge and/or suppress ROS or free radicals, therefore interfering with the oxidation process. These enzymes (superoxide dismutase, glutathione) are endogenously present in humans and are engaged in defence mechanisms. When this mechanism fails to combat ROS, exogenous antioxidants are required, which can be obtained naturally from medicinal plants [4]. Numerous plants have been reported for their antioxidant potential and are known to contain secondary metabolites such as flavonoids, terpenoids, phenols, tannins, etc which shows beneficial antioxidant effects.

Annona muricata L, often known as soursop, graviola, or guanabana, is a member of the Annonaceae family. It can be found in tropical and subtropical regions of the world, such as India, Malaysia, and Nigeria. It is an essential medicinal plant that is widely used in traditional medicine to treat a wide range of human maladies and disorders, including cancer, neuralgia, arthritis, diarrhoea, dysentery, fever, parasites, rheumatism, skin rashes, diabetes, headaches, and sleeplessness [11]. The goal of this study is to see how effective different Annona

muricata L. extracts are as antioxidants.

2. MATERIAL AND METHOD

i) DPPH Radical Scavenging Assay

The DPPH radical scavenging properties of the test plant extracts were measured using the method reported by Smith et al, 1987 [10]. In the test tubes, different concentrations of the sample (100-500g) were obtained, and the volume in each test tube was brought up to 1mL with methanol. 3mL of DPPH solution (with an absorbance of 1) was added to each tube and incubated in the dark for 15 minutes. The absorbance was measured spectrophotometrically at 517nm after incubation, using methanol as a blank. The formula was used to calculate the percentage inhibition.

Percentage inhibition = [Abs of control – Abs of sample/ abs of control] * 100

ii) ABTS Radical Scavenging Assay

The stable ABTS radical was used to evaluate the free radical scavenging capacity of the test plant extracts, as described by Huang et al, 2011 [6], with minor modifications. Different concentrations of the material (100g - 500g) were placed in test tubes, and the volume in each test tube was increased to 1mL with methanol. 3mL of ABTS solution was added to each tube and incubated in the dark for 30 minutes. The absorbance was measured spectrophotometrically at 734nm after incubation, using methanol as a blank. The formula was used to calculate the percentage inhibition.

Percentage inhibition = [Absorbance of control – Absorbance of sample/ absorbance of control] * 100

iii) Nitric Oxide Scavenging Assay

The test plant extracts' nitric oxide scavenging ability was calculated using the method reported by Usha et al, 2012 [12] with minor modifications. 1 mL of the varied strengths of extracts were combined with 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline and incubated at 25°C for 180 minutes. An equivalent volume of freshly produced Griess reagent was added to the extracts (Griess reagent was prepared by mixing equal amounts of 1 percent sulphanilamide in 2.5 percent phosphoric acid and 0.1 percent naphthylethylene diamine dihydrochloride in 2.5 percent phosphoric acid immediately before use). Gallic acid was employed as a positive control and the absorbance was measured at 546 nm. The following formula was used to compute the percentage inhibition of the extracts and standard:

Percentage inhibition = [Absorbance of control – Absorbance of sample/ absorbance of control] * 100

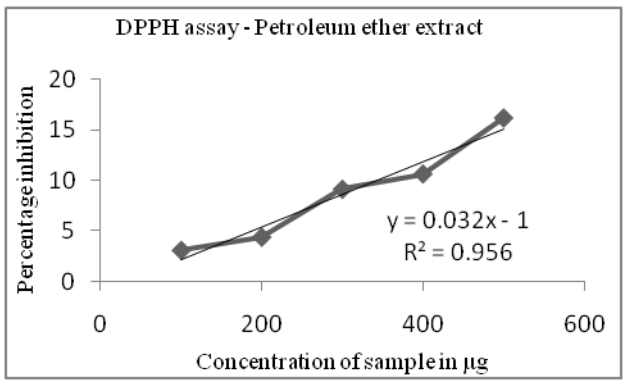
iv) Statistical Analysis

The results are presented as mean standard deviation. The statistical comparison between the groups was done using one way ANOVA and Graph Pad prism at a significance level of 0.05.

3. RESULTS AND DISCUSSION

Various free radicals promoting substances were utilized in the present work to study the antioxidant potential of test plant extracts and results are shown in figures 1-18 and IC 50 values are presented in table 1- 3.

i) DPPH Radical Scavenging Assay



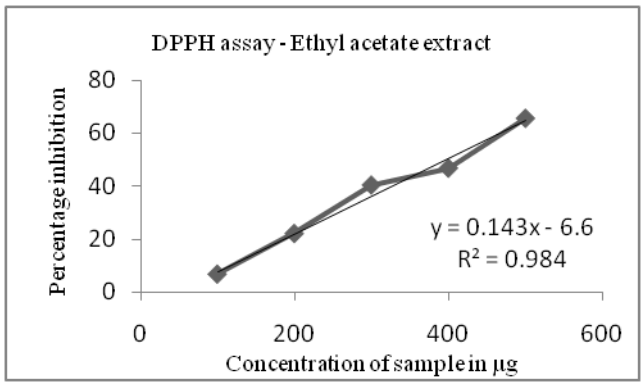
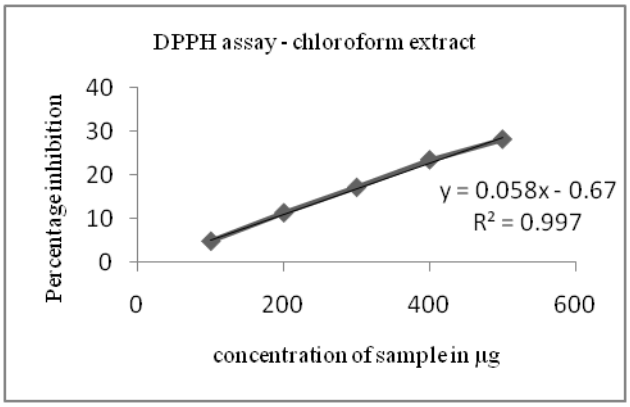


Figure 1: DPPH Radical Scavenging Assay of Petroleum Ether Extract.
Figure 2: DPPH Radical Scavenging ASSAY of Ethyl Acetate Extract.



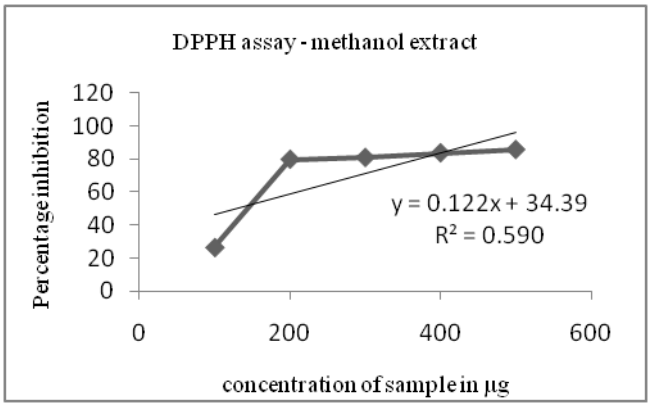
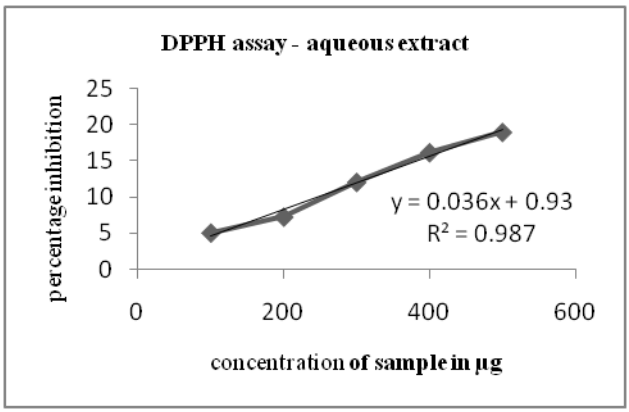


Figure 3: DPPH Radical Scavenging Assay of Chloroform Extract.
Figure 4: DPPH Radical Scavenging Assay of Methanol Extract.



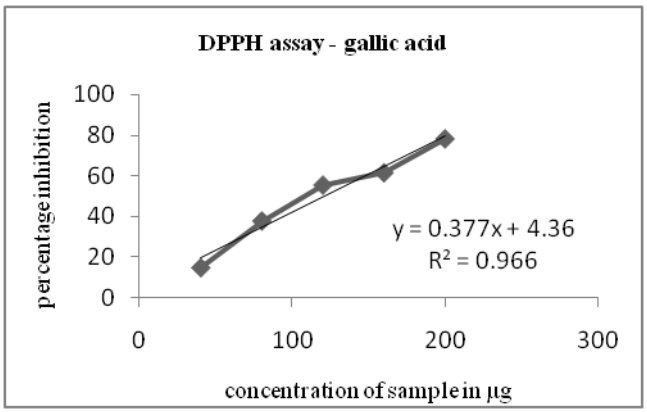
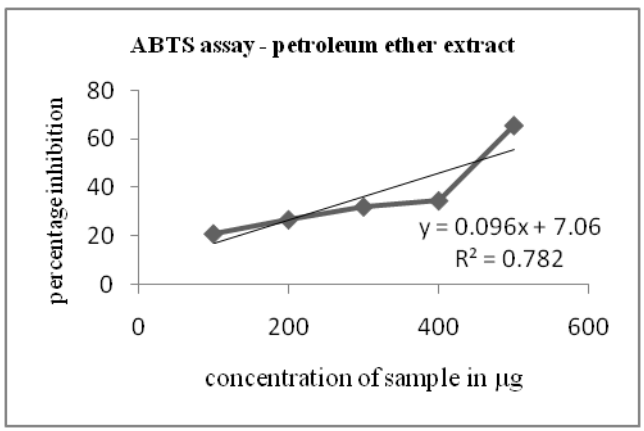


Figure 5: DPPH Radical Scavenging Assay of Aqueous Extract.
Figure 6: DPPH Radical Scavenging Assay of Gallic Acid.

Table 1: DPPH radical scavenging Assay - IC 50 value

Sample (Extracts)	IC50 Value (mg)
Petroleum ether	1.583
Ethyl acetate	0.395
Chloroform	0.863
Methanol	0.127
Aqueous	1.337
Gallic acid (standard)	0.121

ii) ABTS Radical Scavenging Assay



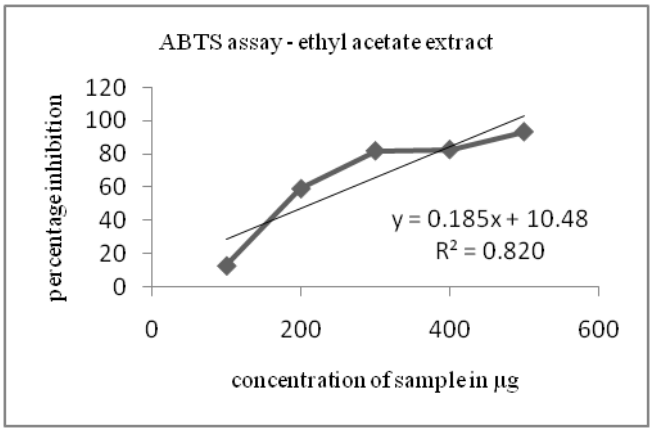
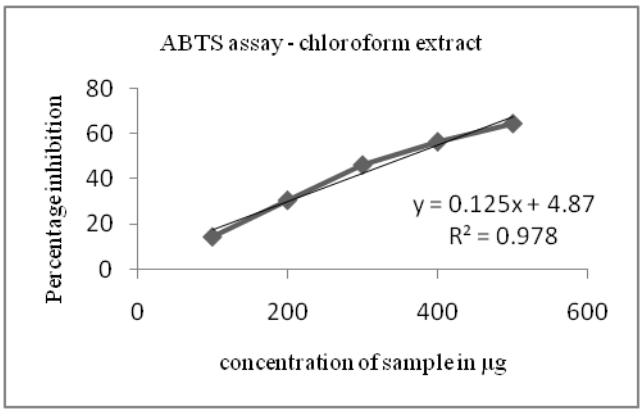


Figure 7: ABTS Radical Scavenging Assay of Petroleum Ether Extract.
Figure 8: ABTS Radical Scavenging Assay of Ethyl Acetate Extract.



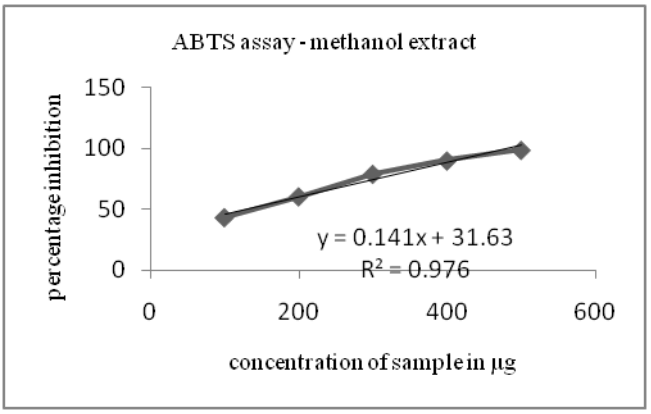
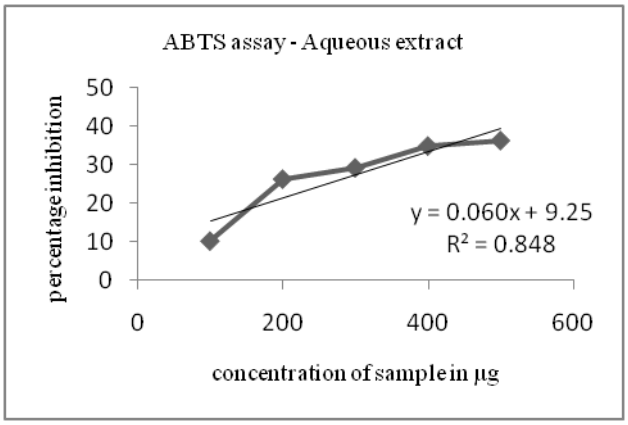


Figure 9: ABTS Radical Scavenging Assay of Chloroform Extract.
Figure 10: ABTS Radical Scavenging Assay of Methanol Extract.



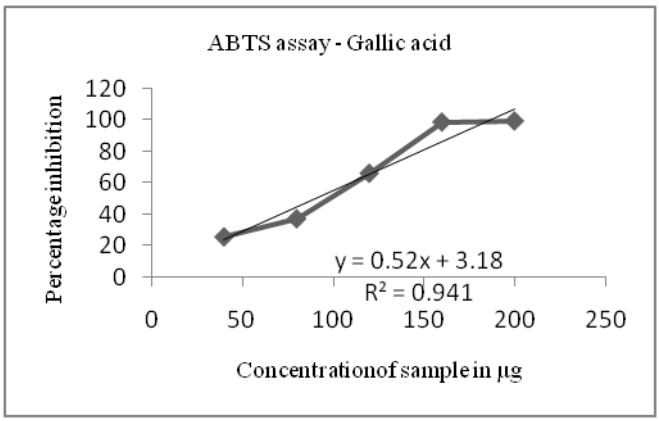
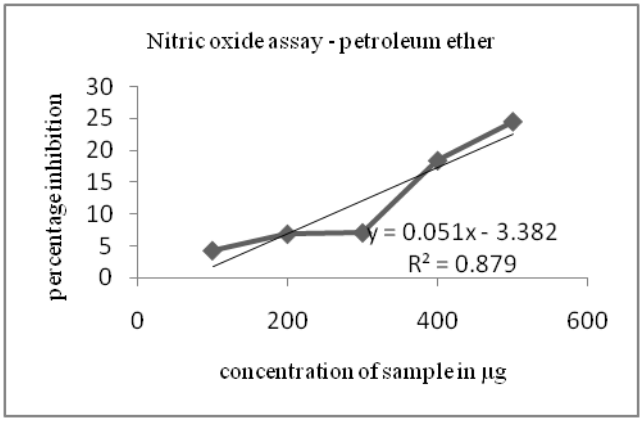


Figure 11: ABTS Radical Scavenging Assay of Aqueous Extract.
Figure 12: ABTS Radical Scavenging Assay of Gallic Acid.

Table 2: ABTS Radical Scavenging Assay - IC 50 Value

Sample (extracts)	IC50 value (mg)
Petroleum ether	0.443
Ethyl acetate	0.213
Chloroform	0.360
Methanol	0.130
Aqueous	0.678
Gallic acid (standard)	0.090

iii) Nitric Oxide Scavenging Assay



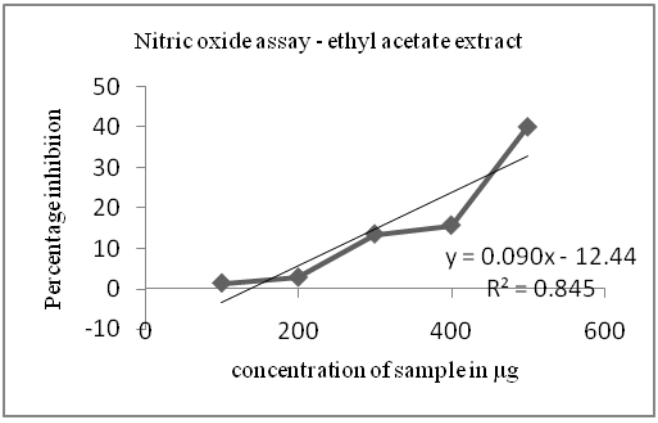
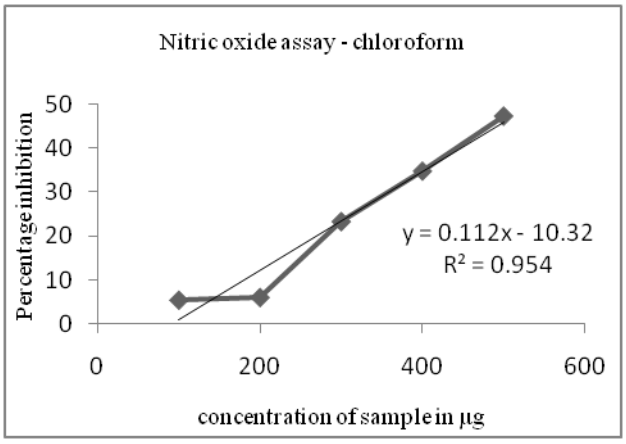


Figure 13: Nitric Oxide Scavenging Assay of Petroleum ether Extract.
Figure 14: Nitric Oxide Scavenging Assay of Ethyl Acetate Extract.



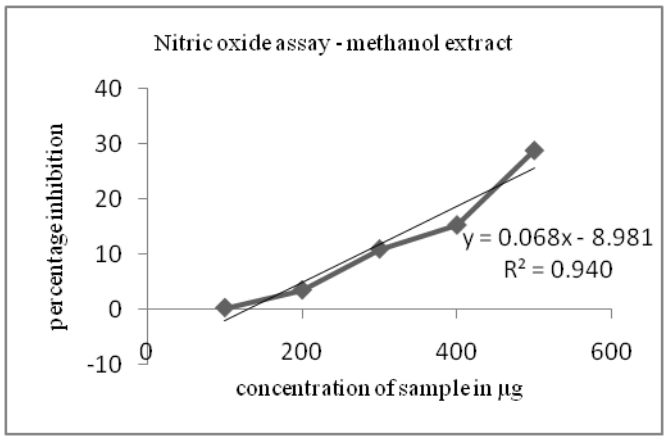
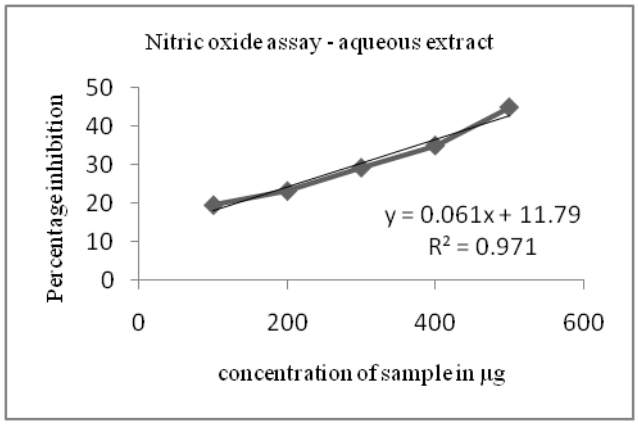


Figure 15: Nitric Oxide Scavenging Assay of Chloroform Extract.
Figure 16: Nitric Oxide Scavenging Assay of Methanol Extract.



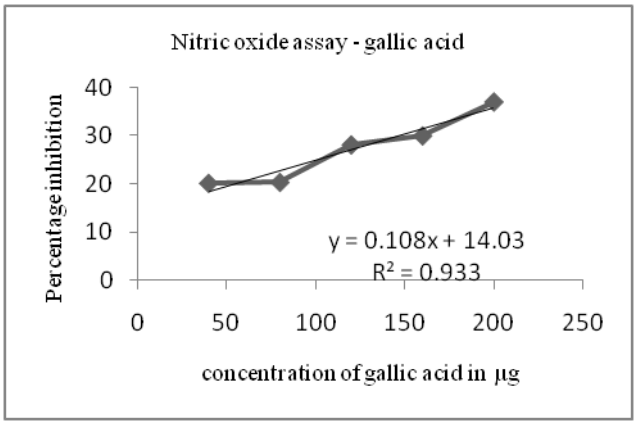


Figure 17: Nitric Oxide Scavenging Assay of Aqueous Extract.
Figure 18: Nitric Oxide Scavenging Assay of Gallic Acid.

Table 3: Nitric Oxide Scavenging Assay - IC 50 Value

Sample (Extracts)	IC50 value (mg)
Petroleum ether	1.030
Ethyl acetate	0.690
Chloroform	0.536
Methanol	0.856
Aqueous	0.617
Gallic acid (standard)	0.332

4. DISCUSSIONS

The subject of antioxidant research is rapidly expanding, with a particular focus on discovering naturally occurring antioxidants to replace synthetic antioxidants, which are being banned due to their carcinogenicity [1]. The radical scavenging ability of plant extracts/compounds is ascribed to their antioxidant capacity. The antioxidant activity of petroleum ether, ethyl acetate, chloroform, methanol, and aqueous extracts of Annona muricata L in vitro was studied using DPPH, ABTS, and nitric oxide radical scavenging activities in the current study. The DPPH and ABTS•+ tests are commonly employed to determine the generation of persistent chromogen compounds, indicating that they have potential antioxidant properties [8]. The approaches have demonstrated that the extracts are more effective than the reference

standard gallic acid.

Methanol extract of *Annona muricata* showed greater DPPH radical scavenging property (85.70%) compared to other extracts. It also showed better ability than the standard used (78.3%). At a concentration of 500 g/ml, petroleum ether extract (16.1 percent) had the lowest activity. Secondary metabolites are thought to be responsible for plant extracts' abilities. These chemicals enable the DPPH radical to be reduced to its 2,2-diphenyl-1-hydrazine form by donating hydrogen atoms, resulting in a shift in colour from purple to yellow and a variable reduction in their absorbance measured at 515 nm [5,9].The DPPH free radical scavenging activity of the *Annona muricata* extracts were found to be in the order of methanol> ethyl acetate>chloroform>aqueous>petroleum ether extract.

When determined for ABTS radical scavenging potential methanol extract resulted in better activity and showed 98.6 % of radical inhibition, whereas standard showed 9.44 % inhibition at 500 µg/ml concentration. Lower activity was observed with aqueous extract. The ABTS free radical scavenging activity of the *Annona muricata L* extracts were found to be in the order of methanol> ethyl acetate> petroleum ether >chloroform>aqueous extract.

The free radical nitric oxide (NO) is important in the prolonging of inflammation and immune responses. Because the creation and release of this radical increases inflammation, plant extracts that function as NO scavengers or NO inhibitors could be employed to reduce the spread of inflammation caused by NO [2]. When compared to other test extracts at the same concentration, chloroform extract inhibited NO more effectively. The radical inhibition of chloroform extract was 47.36 percent, while the norm was 36.91 percent. Annona muricata L extracts were shown to have the following radical scavenging activity: chloroform> aqueous > ethyl acetate> methanol> petroleum ether extract. The result, of the present study showed a dose dependent relationship in the radical scavenging activity. The present study also showed the correlation between each group at $p<0.05$ significant level.

4. CONCLUSIONS

The study imparts the antioxidant abilities of different extracts of *Annona muricata L* to fight deadly diseases by scavenging free radicals. All screened *Annona muricata L* extracts exhibited mild to potent activity, where methanol extract showed significant activity in most of the determined methods. Thus the present study concludes methanol extract of *Annona muricata L* to be a potent source for the isolation of antioxidant compound among the tested extracts.

ACKNOWLEDGEMENT

I am grateful to RGUHS for providing financial assistance under the project no. 19ALL395 (2019-20).

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